Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 16 (3): 749–752
https://doi.org/10.15560/16.3.749

PENSOFT

First report of *Coenosia attenuata* (Stein, 1903) (Diptera, Muscidae) in Uruguay, confirmed by DNA barcode sequences

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Abstract
Coenosia attenuata (Stein, 1903) is a predatory fly which feeds on other insects and can be used as a possible biological control agent. We report this insect in Uruguay for the first time. The flies were found in greenhouses on tomatoes and sweet peppers and identified using both DNA barcoding and morphological characteristics.

Keywords
Biological control, Coenosiinae, COI, horticulture, predator

Academic editor: Kirstern Lica Follmann Haseyama | Received 14 January 2020 | Accepted 4 May 2020 | Published 19 June 2020

Citation: Giambiasi M, Rodríguez A, Arruabarrena A, Buenahora J (2020) First report of *Coenosia attenuata* (Stein, 1903) (Diptera, Muscidae) in Uruguay, confirmed by DNA barcode sequences. Check List 16 (3): 749–752. https://doi.org/10.15560/16.3.749

Introduction

The predatory fly *Coenosia attenuata* (Stein, 1903) (Muscidae, Coenosiinae) is a polyphagous predator, and some of its prey are common pests of greenhouse and outdoor crops. The larvae live in the soil and feed on small soft-bodied invertebrates, such as larvae of Sciaridae and Ephydridae (Diptera) (Mateus 2012). Adults live on the ground and feed on flying insects which generally cause damage to crops, such as *Bemisia tabaci* (Gennadius, 1889), *Trialeurodes vaporariorum* Westwood, 1856, *Bactericera cockerelli* (Šulc, 1909), and *Tuta absoluta* (Meyrick, 1917), among others (Mateus 2012; Martins et al. 2012; Bautista-Martínez et al. 2017). Adults wait for their prey to land on plants or other surfaces or attack prey which fly nearby in an ambush hunting behavior. An interesting behavior of this predator is that it attacks and kills prey even when it is not hungry (Martínez and Cocquempot 2000). Its ability to tolerate high temperatures while maintaining predatory activity makes it especially interesting for biological control of pests in greenhouses and in warm environments (Gilioli et al. 2005).

Coenosia attenuata is native from southern Europe (Hennig 1964). It is currently distributed in several countries of Europe, Asia, Africa, and Oceania (Pohl et al. 2012). In the Americas, this fly was recorded for the first time in Ecuador, Peru, and the United States in 2002 (Martínez-Sánchez et al. 2002; Hoebeke et al. 2003). Later, it was found in Canada (Sensenbach 2004), Colombia (Pérez 2006), Costa Rica (Hernández 2008), Chile (Couri and Salas 2010), Mexico (Bautista-Martínez et al. 2017), Venezuela (Solano et al. 2017), Brazil (Couri et al. 2018), and Honduras (Orozco 2018).

In this work, we report the presence of *Coenosia attenuata* (Stein, 1903) in Uruguay for the first time

by using DNA barcoding and taxonomic identification methods.

Methods

During 2019, we collected specimens of adult flies from tomato and sweet pepper crops from greenhouses in Salto, Uruguay (Fig. 1). The geographical coordinates were obtained using Google Earth® v. 7.3.2 (geodetic datum: WGS84). Morphological identification of the specimens was based on Pohl et al. (2012). The samples were deposited in the Instituto Nacional de Investigación Agropecuaria (INIA), Salto Grande's Insects Collection. The insects used for the DNA barcode were photographed laterally, dorsally, and ventrally and labeled appropriately before DNA extraction.

Total DNA was extracted from individual samples according to a standard CTAB method described by Doyle and Doyle (1987). In this case, after being photographed, the entire body of the fly was used for DNA extraction. The mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified and sequenced using primers LepFoIF RKTCAACMAATCATAAAGATATTGG and COISG-R TAAACTTCTGGRTGWCCAAAAAATCA. The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µl of PCR buffer (with 20 mmol MgCl2), 0.8 units Taq DNA polymerase (Thermo Fisher Scientific, USA), ultrapure water was added for a final volume of 25 µl. Temperature cycling was as follows: 95 °C (4 min), 40 cycles of 95 °C (50 sec), 52 °C (50 sec), 72 °C (50 sec), and a final extension at 72 °C (5 min).

PCR products were purified by the standard method described by Sambrook and Russell (2006) and sent to Macrogen Inc. (Seoul, Korea) sequencing service (http://www.macrogen.com/). The resulting sequences were analyzed using a sequence alignment editor (BioEdit v. 7.2.5; Hall 1999). The taxonomic affinities of our nucleotide sequences were identified using the Basic Local Alignment Search Tool, BLAST (Johnson et al. 2008) and the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). The obtained sequences have been deposited in GenBank and their accession numbers are MN880872 and MN909948.

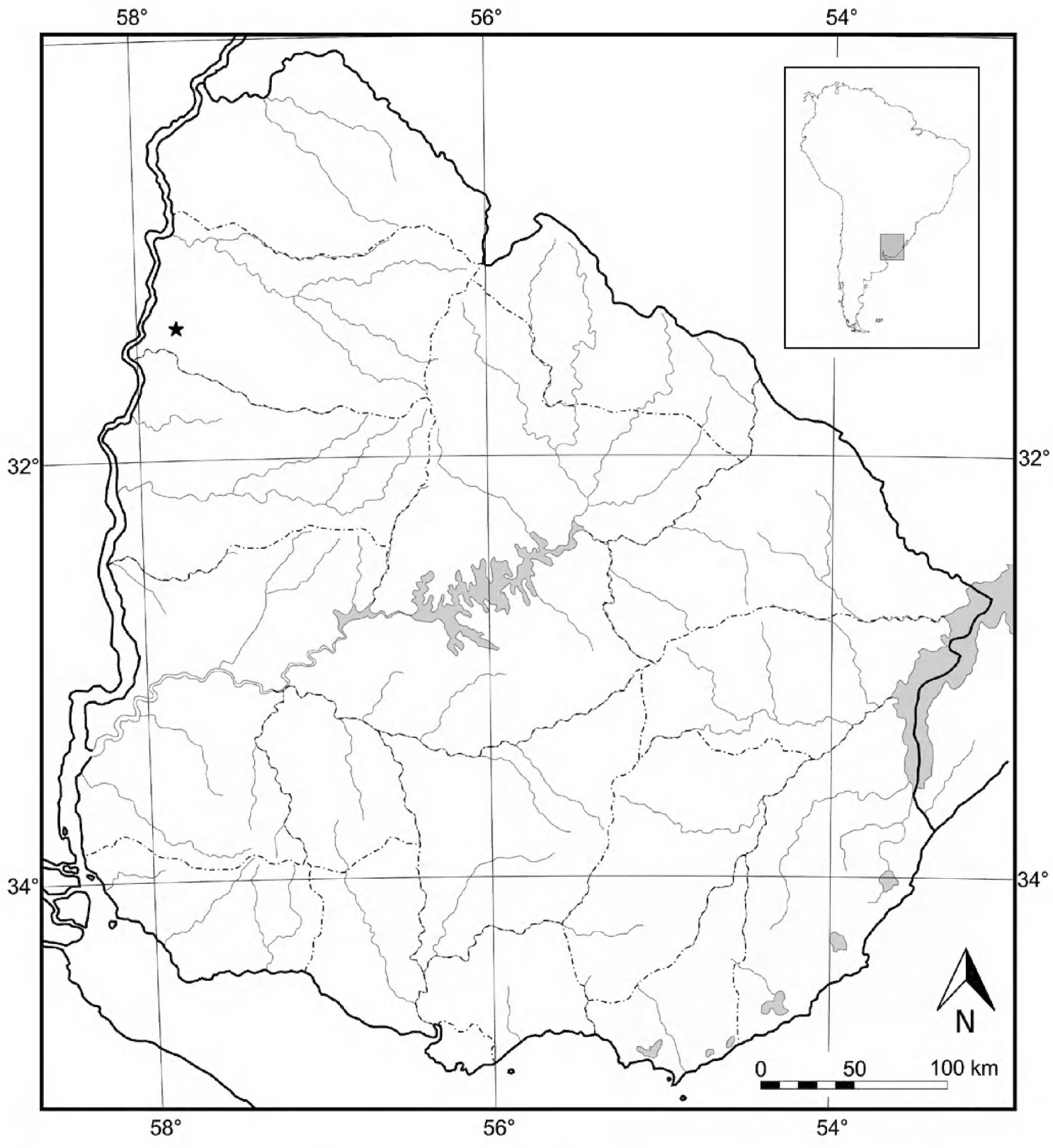

Figure 1. Map of Uruguay and the geographical location of the collection site (star icon) for the first record of *Coenosia attenuata* (Stein, 1903).

Results

Coenosia attenuata (Stein, 1903)

New records. URUGUAY • 1♀; Salto; 31°21′07.3″S, 057° 51′03.4″W; 16 Oct. 2019; Rodríguez leg.; greenhouse with tomato; COI; GenBank MN880872; specimen code MG2_coiSG • 1 ♂; same collection data as for preceding; 31°21′07.8″S, 057°51′00.8″W; greenhouse with sweet pepper; GenBank MN909948; specimen code M4_coiSG.

Identification. The flies are identified as *C. attenuata* (Stein, 1903). All morphological characteristics match the redescription of this species by Pohl et al. (2012). The flies measure approximately 2.5–5.00 mm in length. The female, which is slightly larger and darker than the male, has black antennae, its femora is largely black, and its abdomen is distinct with black bands. The male has yellow antennae. Legs, face, and frons are silvery white and abdomen has slightly pronounced black bands (Fig. 2).

The samples MG2_coiSG, from tomato crop, and M4_coiSG, from sweet pepper crop, were successfully sequenced for the DNA barcode region of COI, with a sequence length of 658 bp (0% ambiguities) with no evidence of stop codons or contamination. Using the BOLD ID Engine, the comparison with the DNA barcode library resulted in a 100% pairwise nucleotide match over 90 other *C. attenuata* specimens housed in BOLD, confirming their primary identification. NCBI BLAST analysis retrieved 76 *C. attenuata* nucleotide sequences showing 100% nucleotide identity to our sequences MN880872 and MN909948.

Discussion

The presence of *Coenosia attenuata* on most continents, its ability to live in high-temperature environments, and its predatory behavior make it an excellent biological control agent (Martínez and Cocquempot 2000; Pohl et al. 2012). In addition, this species adaptable to laboratory conditions and can be bred in captivity for release in commercial crops (Martins et al. 2015).

We have observed *C. attenuata* hunting *Bemisia tabaci* and *Trialeurodes vaporariorum* in sweet pepper and tomato crops. We saw it using the ambush technique to catch its prey in mid-flight, as described by Martínez and Cocquempot (2000). This is an interesting observation, as *Bemisia tabaci*, *Trialeurodes vaporariorum*, and other reported prey are important pests that affect greenhouse and field crops in Uruguay.

As the identification of *C. attenuata* and its prey in the field is difficult, DNA barcoding is a valuable tool for determining predator and prey. This technique allows for

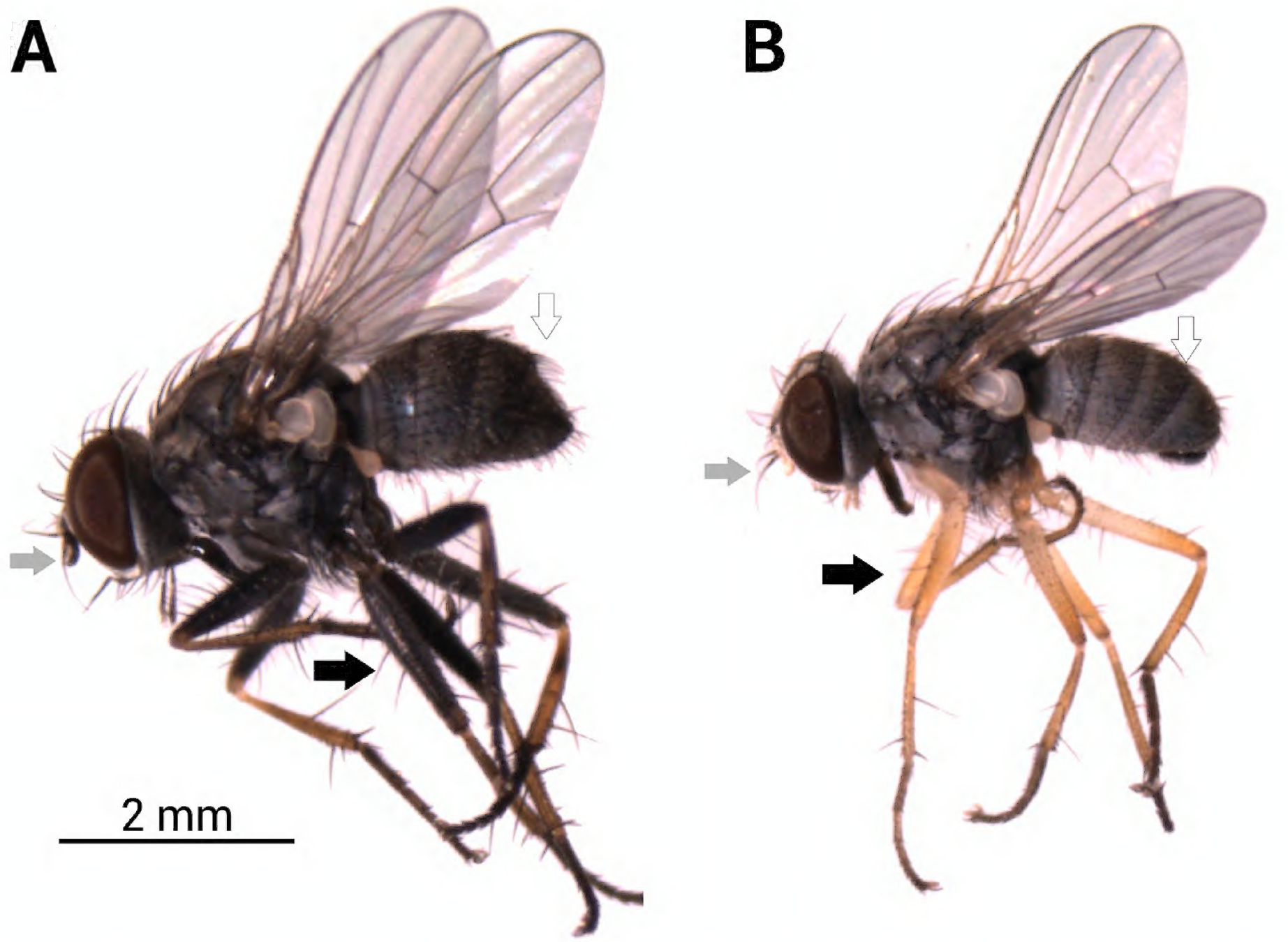

Figure 2. Lateral photograph of *Coenosia attenuata* (Stein, 1903) collected from Uruguay. **A.** Female, MG2_coiSG. **B.** Male, M4_coiSG. Arrows indicate morphological differences between female and male: femur (black), antennae (grey), abdomen color (white). Female individuals are larger.

C. attenuata and other species of insects to be identified with great precision (Orozco 2018). This is highly valuable if included as a routine process when documenting the presence of taxa across their geographical range. It enables rapid and automated species identification of adult or immature individuals with the same precision (Hebert et al 2003).

Acknowledgements

We thank Mariana Cosse for useful comments on barcoding technics and Stephanie Caracciolo for corrections to the English manuscript. We also thank two anonymous reviewers for their constructive criticism on the manuscript. The research for this paper was funded by the Instituto Nacional de Investigación Agropecuaria of Uruguay. The authors have declared that no competing interests exist.

Authors' Contributions

This paper was originally conceived by MG and JB. The study was designed by MG, AA, and JB. MG and AR carried out the fieldwork and processed collected material; MG and AA identified the specimens by barcoding. All authors wrote the final version of the manuscript.

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